

Attorney Docket No.: **T5530.CIP (UT-0006)**
Inventors: **Rao et al.**
Serial No.: **09/109,858**
Filing Date: **July 2, 1998**
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claims 1-11 (canceled)

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Claim 12 (currently amended): A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;

(b) incubating the multipotent CNS stem cells in NEP medium;

(c) replating the multipotent CNS stem cells on laminin in NEP medium in the absence of chick embryo extract to induce cell differentiation;

(d) removing A2B5+ cells from the differentiating cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(e) purifying from the supernatant following step (d) a subpopulation of cells expressing embryonic neural cell adhesion molecules via a procedure selected from the group

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consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, wherein said procedure uses an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule (NCAM); and

(f) incubating the purified subpopulation of cells in a FGF-containing medium ~~configured for supporting adherent growth thereof~~ to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, ~~wherein said neuron restricted precursor cells are capable of differentiating into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte promoting medium containing FGF and 10% fetal calf serum.~~

Claims 13-14 (canceled)

Claim 15 (currently amended): The method of claim 12 wherein ~~said procedure is the subpopulation of cells expressing embryonic neural cell adhesion molecules is purified by specific antibody capture.~~

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Claim 16 (currently amended): The method of claim 12 wherein mammalian the rodent or human multipotent CNS stem cells are neuroepithelial cells.

Claims 17-20 (canceled)

Claim 21 (currently amended): A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube;

(b) dissociating cells comprising the sample of spinal cord tissue removed from the embryo;

(c) removing A2B5+ cells from the dissociated cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(d) purifying from the supernatant following step (c) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic neural cell

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adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule;

(e) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a FGF-containing medium ~~configured for supporting adherent growth of the neuron restricted precursor cells~~; and

(f) incubating the plated cells ~~at a temperature and in an atmosphere conducive to growth in the FGF-containing medium~~ to obtain an isolated, pure population of neuron-restricted precursor cells, ~~wherein said neuron restricted precursor cells are capable of differentiating into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte promoting medium containing FGF and 10% fetal calf serum.~~

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Claims 22-23 (canceled)

24. (amended) The method of claim 21 wherein ~~said procedure is the subpopulation of cells expressing embryonic neural cell adhesion molecules is purified by~~ is specific antibody capture.

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Claim 25 (canceled)

Claim 26 (currently amended): A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 12, ~~wherein said neuron-restricted precursor cells require FGF to support adherent growth, differentiate into ENS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte promoting medium containing FGF and 10% fetal calf serum.~~

Claim 27 (currently amended): A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 21, ~~wherein said neuron-restricted precursor cells require FGF to support adherent growth, differentiate into ENS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte promoting medium containing FGF and 10% fetal calf serum.~~

Claims 28-59 (canceled)

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Claim 60 (currently added): A method of isolating a pure population of mammalian CNS neuron-restricted precursor cells comprising the steps of:

(a) plating mammalian embryonic stem cells in neural differentiation conditions so that the ES cells alter their morphology and express neuronal and glial markers nestin, NCAM, MAP2 kinase, GFAP and cyclophilin/DM20/PLA;

(b) removing A2B5+ cells from the differentiated cells of step (a) via specific antibody capture with an antibody that specifically recognizes A2B5;

(c) purifying from supernatant from step (b) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule;

(d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a FGF-

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containing medium; and

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(e) incubating the plated cells in the FGF-containing
medium to obtain an isolated, pure population of mammalian CNS
neuron-restricted precursor cells.
